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S,S-DI-2-PYRIDYL DITHIOLOPHOSPHATE AS A KEY INTERMEDIATE IN THE PHOSPHORYLATION BY OXIDATION-REDUCTION CONDENSATION

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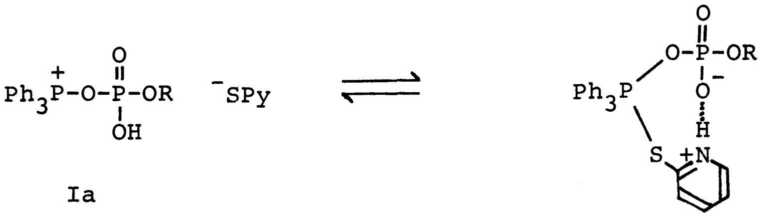
A key intermediate of phosphorylation by oxidation-reduction condensation which is useful for the preparation of various nucleotides was studied by means of ^{31}P NMR using methanephosphonate as a model compound. The intermediate, S,S-di-2-pyridyl methanedithiolophosphonate, was formed quite rapidly by the reaction of methanephosphonic acid and triphenylphosphine-d_{15}-2,2'-dithiodipyridine $(PyS)_2$ in anhydrous pyridine and could be stored in solution.

It has been reported in previous papers that the use of triphenylphosphine (Ph_3P) and 2,2'-dithiodipyridine $(PyS)_2$ as the coupling reagents led to the formation of biologically active peptides, such as LH-RH[1], ACTH (1-24)[2], and oligonucleotides, cyclic phosphates of nucleoside[3] and Coenzyme A[4] in good yields. In the phosphorylation reaction, the dehydration between monoesters of phosphoric acid and nucleophilic components proceeds by eliminating one oxygen atom with Ph_3P (reduction) and two hydrogen atoms with $(PyS)_2$ (oxidation) to afford mixed esters of phosphoric acid, $Ph_3P{=}O$ and 2 mol of 2-pyridinethione.

This paper describes the structure of an active intermediate in the phosphorylation by oxidation-reduction condensation.

It seems necessary that the key intermediate exists[5] as a comparatively stable one to exclude the formation of symmetrical pyrophosphate for application to the selective phosphorylation of alcohol, amine or phosphoric acid. In the oxidation-reduction condensation, it was also found that an active intermediate (I) was formed quite rapidly and could be stored in solution when 3'-O-acetylthymidine 5'-phosphate was allowed to react with excess Ph_3P and $(PyS)_2$ in a small amount of pyridine. This was indicated by the following findings, though (I) was not isolated. First, sym-pyrophosphate was no longer produced even though the solution of I was further diluted with pyridine. Second, thymidine 5'-phosphate (pT), a starting material, was recovered quantitatively by hydrolysis of I. Third, thymidine 5'-phosphoromorpholidate or 5'-O-tritylthymidine-(3' → 5')-3'-O-acetylthymidine was produced by the addition of morpholine or 5'-O-trithylthymidine (TrT), respectively, to the solution of I.

Examination about the stability of I by TLC and paper electrophoresis showed no detectable decomposition after 1 day at r.t. Further, paper chromatography showed that 90% of pT was obtained along with 10% sym-pyrophosphate after the solution of I had been kept standing for 60 hr at r.t., followed by mild alkaline hydrolysis. In the preceding reports a structure of the intermediate was tentatively described as phosphinyloxyphosphonium salt (Ia) which is stabilized by the formation of intramolecular hydrogen bond as shown in the scheme.


Ia

Ib

To elucidate the structure of I, ^{31}P NMR spectra of the reaction mixture were studied. At first, pT-OAc was treated with 3 equiv each of $Ph_3P\text{-}d_{15}$ and $(PyS)_2$ in anhydrous pyridine. The spectrum of the reaction mixture showed the formation of a new compound A which showed a singlet with δ =-45.5 ppm as shown in Fig 1.

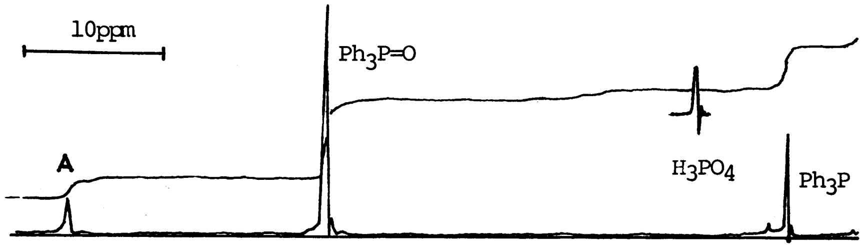
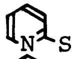
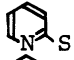
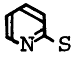
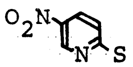


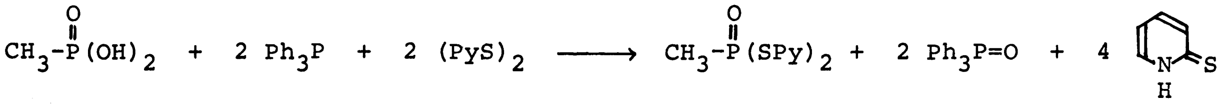
Fig 1. ^{31}P NMR spectra of the reaction mixture after treatment of pT-OAc with 3 equiv each of Ph_3P and $(PyS)_2$ in anhydrous pyridine

This great low field shift implies the presence of P-S linkage, such as S-pyridyl-5'-O-thymidylthiophosphate (II) a possible intermediate. But, II can be obtained only by treating I with strong base in the presence of large excess of 2-pyridinethione and succeeding hydrolysis. In order to make determination of the exact structure and the property of I, pT is not convenient because of its low phosphorus atom content and low solubility. Then, methanephosphonic acid (III) was selected as a model compound and ^{31}P NMR spectra of its derivatives were studied. ^{31}P NMR shift data for the reaction mixture of III with various tertiary phosphine and 2,2'-dithiodipyridine (molar ratio 1 : 3 : 3) in anhydrous pyridine was shown in Table 1. As it can be seen in Table 1, chemical shifts hardly change depending on the structure of the phosphines (Exp. No. 1-3), but a little change was observed when nitro group is introduced in the 5 position of the pyridine ring (Exp. No. 4)

Table 1. ^{31}P NMR data for the reaction intermediate of III with R_3P and $(R'S)_2$

Exp. No.	R	R'S	δ ppm (ref. 85% H_3PO_4)
1	C_6H_5		$-56.0\,(^2J_{PH} = 14.4\ Hz)$
2	$p\text{-}CH_3O\text{-}C_6H_4$		$-55.7\,(^2J_{PH} = 14.2\ Hz)$
3	$n\text{-}C_4H_9$		$-55.6\,(^2J_{PH} = 14.1\ Hz)$
4	C_6H_5		$-55.0\,(^2J_{PH} = 14.0\ Hz)$

These values are adequately approximate to a reported value of $CH_3\text{-}\overset{O}{\overset{\|}{P}}(SBu)_2$, -53.3 ppm.[6] From ^{31}P NMR, it was also found that at least 2 mol of Ph_3P were employed for the formation of I. Consequently, the structure of I is estimated to be S,S-di-2-pyridyl methanedithiolophosphonate (Ic) shown in the following equation.



Ic

This compound was separately prepared by the reaction of $CH_3\text{-}\overset{O}{\overset{\|}{P}}Cl_2$ with 2 mol of 2-pyridinethione in anhydrous pyridine and ^{31}P NMR spectrum of the mixture showed clear quartet centered at -55.6 ppm ($^2J_{PH}$ = 14.6 Hz) as shown in Fig 3.

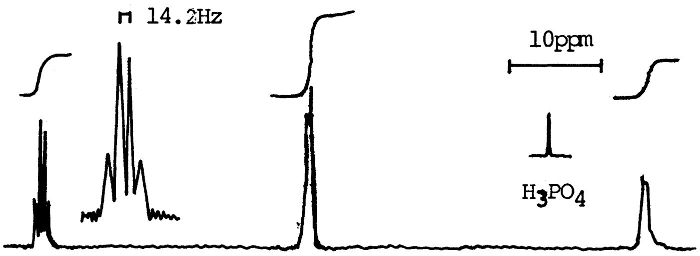


Fig 2. ^{31}P NMR spectra of the reaction mixture after treatment of $CH_3\text{-}\overset{O}{\overset{\|}{P}}(OH)_2$ with 3 equiv each of $(p\text{-}CH_3O\text{-}C_6H_4)_3P$ and $(PyS)_2$

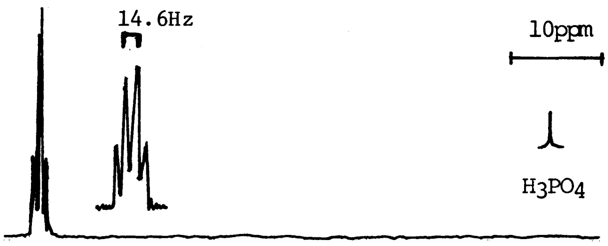
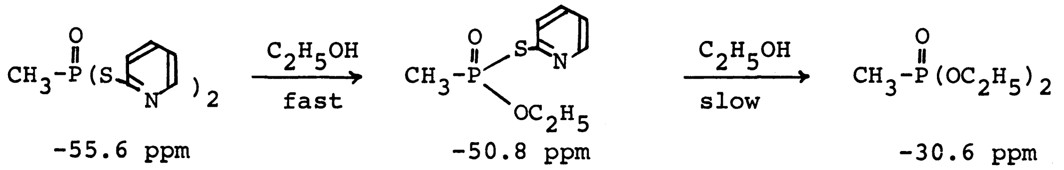
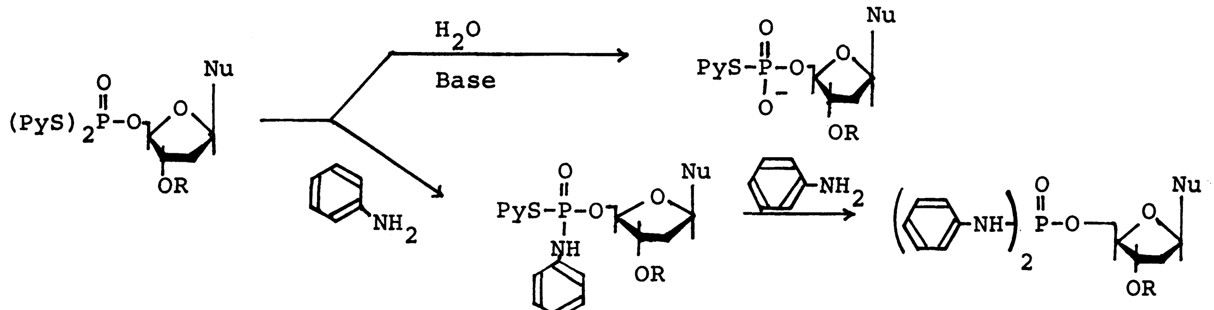


Fig 3. ^{31}P NMR spectrum of the reaction mixture after treatment of $CH_3\overset{O}{\overset{\|}{P}}Cl_2$ with 2 mol of 2-pyridinethione

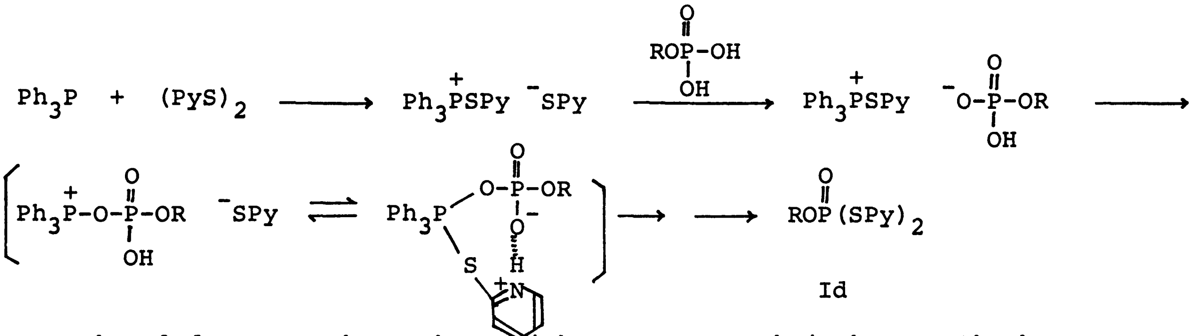
Addition of ethanol to the reaction mixture gradually diminished the signal at -55.6 ppm and new peak at -50.8 ppm appeared. After being kept standing over night, another signal at -30.6 ppm appeared. The latter signal was assigned as $CH_3\text{-}\overset{O}{\overset{\|}{P}}(OEt)_2$, -29.6 ppm, in comparison with authentic sample prepared according to the literature method.[7] These changes may be attributed to the stepwise alcoholysis shown in the following equation.



In addition, the following experimental results support the above mentioned mechanism; first, S-2-pyridyl 5'-O-thymidylthiophosphate (II), which is difficult to be prepared by one step procedure from thymidine 5'-phosphate and Ph_3P and $(PyS)_2$, can be obtained as its ammonium salts by hydrolysis of I in the presence of tertiary amines. While, in the absence of amines, pT-OAc and AcO-TppT-OAc are formed by the rapid hydrolysis of II produced. Second, nucleoside 5'-phosphorodianilidate could not be obtained from the reaction of nucleoside 5'-phosphoromonoanilidate and Ph_3P and $(PyS)_2$.[8] But, the dianilidate was produced in good yield directly by the reaction of nucleoside 5'-phosphate with 5 equiv each of Ph_3P and $(PyS)_2$ in the presence of excess amount of aniline.[9]



Based on these results, the phosphorylation by the oxidation-reduction condensation proceeds, at least in anhydrous pyridine, through an S,S-di-2-pyridyl dithiolophosphate (Id) intermediate as shown in the following equation. As mentioned above, the first pyridylthio group of Ic is easily exchanged by nucleophilic function, therefore, the triester type intermediate is considered to be quite useful for the phosphorylation in preparing various nucleotide derivatives.



Id

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